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Note

Fmoc-peptide acid chlorides : Preparation, characterization and utility in peptide synthesis

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Fmoc-protected peptide acid chlorides have been prepared as crystalline solids and characterized. They are used as coupling agents for the synthesis of the model tetrapeptide, Leu-Ala-Gly-Val.

Although the powerful activation present in acid chlorides was well known by the turn of the century when the first attempts at peptide bond formation were made by Emil Fisher[1], both preparation and application of amino acid chloride method were stated to be far from unequivocal and obsolete[2,3]. This position was altered dramatically by the important discovery that Fmoc-amino acid chlorides are stable convenient intermediates[4,5]. A revised view of acid chlorides in peptide chemistry is being brought by the development of mild procedures for peptide bond formation. The potassium salt of 1-hydroxybenzotriazole (KOBt) was found to be an efficient coupling additive[6]. It was used successfully to accomplish rapid and efficient acylation reactions employing Fmoc-amino acid chlorides. The coupling was found to be free from racemization[7,8]. The *C*-methylene doublets and methyl ester singlets of the NMR spectra[9] of the protected diastereomeric dipeptides, Fmoc-Phg-Phe-OMe and Fmoc-D-Phg-Phe-OMe as well as that of Fmoc-MePhg-Phe-OMe and Fmoc-D-MePhg-Phe-OMe, prepared by using KOBt as coupling agent, revealed that the coupling process is free from racemization. Similar results have been observed in the case of Bz-Phg-Ala-OMe and Bz-D-Phg-Ala-OMe which were made by solid phase method using KOBt. No additional base was used. Thus, the synthesis of [Leu]enkephalin, β-casomorphin and cyclosporine fragments 4-8 and 8-11 have been accomplished. However, no attempts have been made so far to prepare peptide acid chlorides[10]. The present paper describes the preparation and utility of N^{α}-Fmoc-protected peptide acid chlorides in peptide synthesis.

It is important to note that activation in the form of acid azides has been in use as a powerful tool in peptide synthesis. Inspite of several known side reactions associated with the formation and use of peptide hydrazides and azides, acylpeptide azides have been employed for the coupling. It is found that N^{α}-Fmoc protected peptide acid chlorides can be conveniently prepared by stirring the mixture of Fmoc-peptide acids with thionyl chloride in dichloromethane under anhydrous conditions for 24 hr. The course of the reaction can be monitored on TLC using the solvent system ethyl acetate-hexane (35:65). Table I lists the Fmoc-di- and tri-peptide acid chlorides prepared to date. All peptide acid chlorides have been isolated as crystalline solids which can be stored for long periods under anhydrous conditions. They have been obtained in reasonable yields and purity. The purity of peptide acid chlorides was checked by HPLC by converting them to their methyl esters and injecting onto a C-18 reverse phase Deltapak column (15μ, 100 A°, 3.9 x 300 mm).

Fmoc-Cl[11], Fmoc-amino acids[12], Fmoc-amino acid chlorides[4,5] and KOBt[7] were prepared following the reported procedures. Fmoc-protected dipeptide methyl esters have been prepared by coupling Fmoc-amino acid chlorides to amino acid methyl ester hydrochloride salts in dichloromethane in the presence of KOBt. They were subjected to base hydrolysis using 0.1 *N* NaOH in methanolic medium and the corresponding carboxyl free Fmoc-dipeptide acids were isolated as crystalline solids.

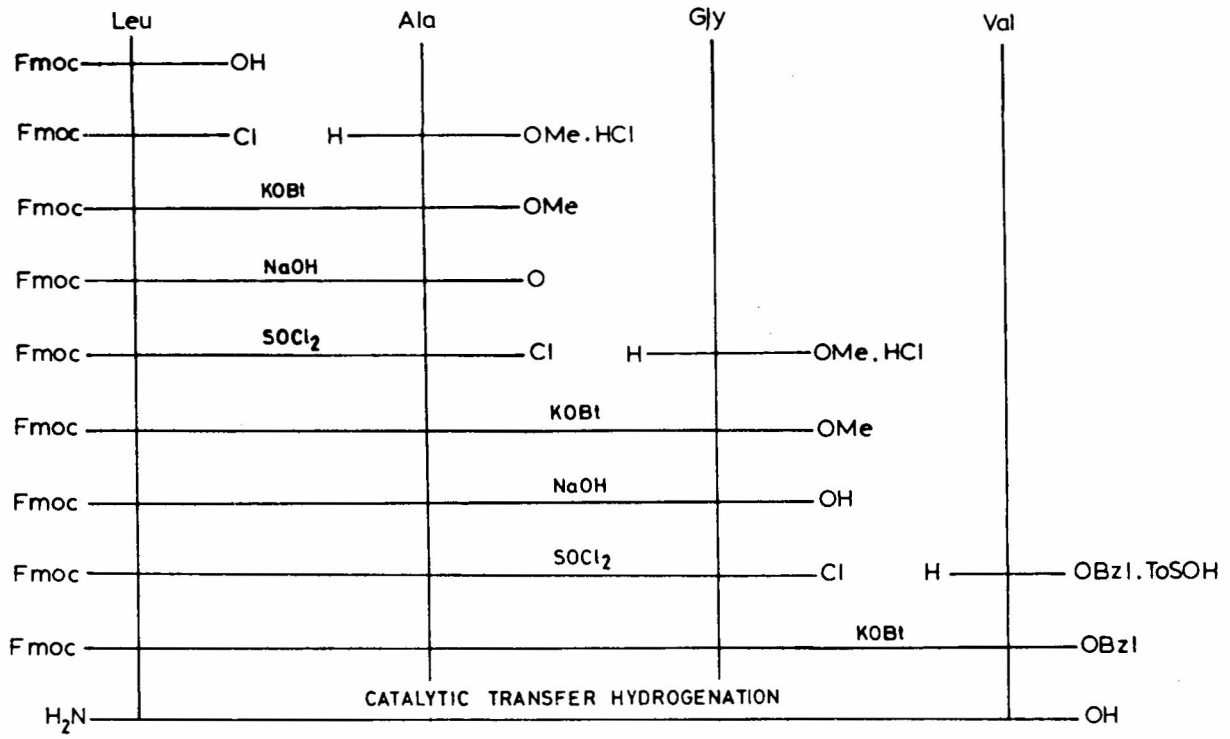
Fmoc-peptide acid chlorides have been used as coupling agents for the synthesis of peptides by extending the peptide chain from *N*-terminal to *C*-

Table I—Fmoc protected peptide esters, acids and acid chlorides*

Sl. No.	Name of the Peptide	Yield (%)	m.p. (°C)	R_f value R_fA*	R_fB##	R_fC##	$[\alpha]_D^{25}$	Mol. formula	Found % (Calcd) C	H	N
1	Fmoc-Leu-Ala-Cl	72	65-68	0.66	—	0.70	+ 40° (c 0.5, $CHCl_3$)	$C_{24}H_{27}N_2O_4Cl$	—	—	—
2	Fmoc-Leu-Ala-OMe	80	162-63	0.64	0.48	—	- 30° (c 0.5, $CHCl_3$)	$C_{25}H_{30}N_2O_5$	68.09 (68.47	6.82 6.87	6.08 6.38)
3	Fmoc-Leu-Ala-OH	82	148-50	0.56	0.40	—	+ 80° (c 0.5, $CHCl_3$)	$C_{24}H_{28}N_2O_5$	68.4 (67.90	6.72 6.65	6.71 6.60)
4	Fmoc-Tyr(Bzl)-Pro-Cl	69	94-96	0.66	—	0.70	- 15.5° (c 1, $CHCl_3$)	$C_{29}H_{27}N_2O_5Cl$	—	—	—
5	Fmoc-Tyr(Bzl)-Pro-OMe	79	200-202	0.67	0.39	—	+ 77.7° (c 1, $CHCl_3$)	$C_{30}H_{30}N_2O_6$	69.82 (70.07	5.81 5.87	5.53 5.44)
6	Fmoc-Tyr(Bzl)-Pro-OH	70	112-14	0.63	0.79	—	- 26.6° (c 0.5, $CHCl_3$)	$C_{29}H_{28}N_2O_6$	69.38 (69.59	5.72 5.64	6.31 5.60)
7	Fmoc-Pro-Pro-Cl	78	140-41	0.61	—	0.67	+ 30.2° (c 1, $CHCl_3$)	$C_{25}H_{25}N_2O_4Cl$	—	—	—
8	Fmoc-Pro-Pro-OMe	75	Gum	0.65	0.71	—	+ 40.1° (c 1, $CHCl_3$)	$C_{26}H_{28}N_2O_5$	69.59 (69.62	6.34 6.29	6.31 6.24)
9	Fmoc-Pro-Pro-OH	76	132-33	0.51	0.56	—	+ 20.1° (c 1, $CHCl_3$)	$C_{25}H_{26}N_2O_5$	68.84 (69.10	5.94 6.03	6.53 6.44)
10	Fmoc-Ala-Leu-Cl	76	114-16	0.55	—	0.69	+ 18.1° (c 1, $CHCl_3$)	$C_{24}H_{27}N_2O_4Cl$	—	—	—
11	Fmoc-Ala-Leu-OMe	82	125-27	0.67	0.72	—	- 30.2° (c 1, $CHCl_3$)	$C_{25}H_{30}N_2O_5$	68.52 (68.47	6.92 6.89	6.42 6.58)
12	Fmoc-Ala-Leu-OH	74	215-17	0.50	0.56	—	- 25.1° (c 1, $CHCl_3$)	$C_{24}H_{28}N_2O_5$	67.89 (67.90	6.62 6.64	6.72 6.60)
13	Fmoc-Leu-Ala-Gly-Cl	82	76-78	0.62	—	0.74	+ 18.2° (c 0.5, $CHCl_3$)	$C_{26}H_{30}N_3O_5Cl$	—	—	—
14	Fmoc-Leu-Ala-Gly-OMe	80	92-95	0.75	0.34	—	+ 55.5° (c 0.5, $CHCl_3$)	$C_{27}H_{30}N_3O_6$	65.41 (65.43	6.68 6.71	8.01 8.47)
15	Fmoc-Leu-Ala-Gly-OH	75	118-20	0.48	0.37	—	- 28.2° (c 0.5, $CHCl_3$)	$C_{26}H_{31}N_3O_6$	64.91 (64.85	6.32 6.49	8.79 8.72)
16	Fmoc-Tyr(Bzl)-Pro-Phe-Cl	75	128-29	0.68	—	0.78	+ 40.0° (c 0.5, $CHCl_3$)	$C_{38}H_{36}N_3O_5Cl$	—	—	—
17	Fmoc-Tyr(Bzl)-Pro-Phe-OMe	74	110-12	0.60	0.83	--	+ 127.2° (c 1, $CHCl_2$)	$C_{39}H_{39}N_3O_7$	70.80 (70.78	5.99 5.94	6.29 6.34)
18	Fmoc-Tyr(Bzl)-Pro-Phe-OH	80	80-83	0.56	0.75	--	+ 33.3° (c 0.5, $CHCl_2$)	$C_{38}H_{37}N_3O_7$	70.92 (70.98	5.71 5.80	6.69 6.53)

*The coupling of Fmoc-amino acid chlorides and Fmoc-dipeptide acid chlorides has been carried out in the presence of KOBt in dichloromethane **R_fA = chloroform - methanol - acetic acid (40:2:1); #R_fB = chloroform - methanol (9:1); ##R_fC = ethyl acetate - hexane (35:65); Abbreviations are in accordance with the recommendations of the IUPAC-IUB commission on Biochemical Nomenclature published in 'Pure and Applied Chemistry", 40(1974) 314 and all amino acids used, expect Gly, are of L - configuration.

terminal end following the stepwise strategy. Thus, the synthesis of the model tetrapeptide Leu-Ala-Gly-Val has been accomplished by this method (Scheme I). For this, Fmoc-Leu-Ala-Cl and Fmoc-Leu-Ala-Gly-Cl, prepared from their corresponding protected peptide acids after hydrolysis of their methyl esters using 0.1 *N* NaOH, have been coupled with Gly-OMe.HCl and Val-OBzl.TosOH respectively to obtain the protected tetrapeptide, Fmoc-Leu-Ala-Gly-Val-OBzl, m.p. 129-31°C, R_f (A) 0.43; R_f (B) 0.72; $[\alpha]_D^{25}$ + 40 (c 0.5, $CHCl_3$). It was deprotected using Pd-C/85% HCOOH to obtain Leu-Ala-Gly-Val, 141-43°C; R_f [n-butanol-acetic acid-water (4:1:1, v/v] 0.33; R_f [n-butanol-acetic acid-water (4:1:5 v/v, upper phase) 0.35]; $[\alpha]_D^{25}$ + 21.8° (c 1, ethanol); [Found: C, 53.42; H, 8.2; N, 15.82. Calc. for $C_{16}H_{30}N_4O_5$ (358.72): C,53.57, H,8.43; N,15.69%]; Amino acid analysis [amino acid (Calc.), Found]: Leu(1) 0.98, Ala(1) 0.96, Gly(1) 0.96 and Val(1) 1.01. The purity of peptides, Fmoc-Leu-Ala-Cl and Fmoc-Leu-Ala-Gly-OH was checked by HPLC. The final free peptide acid was made by subjecting the protected peptide to catalytic transfer hydrogenation using Pd black and 85% formic acid. The purity of Leu-Ala-Gly-Val, as checked by HPLC [Waters C18



Scheme I : Synthesis of Leu - Ala - Gly - Val starting with the N-terminal residue (N→C Strategy)

deltapak column (3.9 mm x 30 cm, 15 μ, spherical); flow rate: 1 mL/min; UV monitoring at 210 nm; eluant 60% CH_3CN-H_2O containing 0.1% trifluoroacetic acid; R_t 4.8], is found to be satisfactory.

Thus, Fmoc-protected peptide acid chlorides can be prepared, isolated and used for the stepwise synthesis of peptides extending the chain from *N*- to *C*-terminal end. This strategy is particularly useful for the incorporation of sterically hindered amino acid residues into peptide chains and for the synthesis of peptides by segment coupling.

Experimental Section

Preparation of Fmoc-protected peptide acid chlorides : General procedure. A suspension of Fmoc-peptide acid (1 mmole) in 5 mL of dichloromethane was treated with freshly distilled thionyl chloride (1.5 mmoles) and the mixture was stirred for about 24 hr at room temperature under dry conditions. Evaporation *in vacuo*, followed by the addition of dichloromethane and reevaporation gave a solid free of excess of thionyl chloride. The residue was dissolved in minimum amount of dichloromethane and hexane was added, the resulting crystals were filtered and dried.

General procedure for coupling employing KOBt. To a solution of amino acid ester salt (1 mmole) and KOBt in dichloromethane (3 mL) was added a solution of the Fmoc-peptide acid chloride (1 mmole) and KOBt (1 mmole) in dichloromethane (3 mL) and the mixture was stirred for 30 min at room temperature (reaction monitored on TLC). A 30 mL of dichloromethane was added to the reaction mixture and then washed thrice with 30 mL portions of 5% $NaHCO_3$, 5% HCl, water, saturated NaCl solution and dried. The organic layer was evaporated to give an oil residue which was recrystallised from dichloromethane-hexane to obtain the product in good yield.

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